

Benomyl/Carbendazim

• Intended Use

For detection of benomyl (as carbendazim) and carbendazim in water (groundwater, surface water, well water). Both compounds are measured and reported as ppb carbendazim. For quantitative determination of benomyl, hydrolyze to carbendazim before assaying. For soil, crop, and food use refer to specific application bulletins.

• Principle

The Benomyl/Carbendazim RaPID Assay[®] applies the principles of enzyme linked immunosorbent assay (ELISA) to the determination of carbendazim. The sample to be tested is added, along with an enzyme conjugate, to a disposable test tube, followed by paramagnetic particles with antibodies specific to carbendazim attached. Both the carbendazim (which may be in the sample) and the enzyme labeled carbendazim (the enzyme conjugate) compete for antibody binding sites on the magnetic particles. At the end of an incubation period, a magnetic field is applied to hold the paramagnetic particles (with carbendazim and labeled carbendazim analog bound to the antibodies on the particles, in proportion to their original concentration) in the tube and allow the unbound reagents to be decanted. After decanting, the particles are washed with Washing Buffer.

The presence of carbendazim is detected by adding the enzyme substrate (hydrogen peroxide) and the chromogen (3,3',5,5'-tetramethylbenzidine). The enzyme-labeled carbendazim analog bound to the carbendazim antibody catalyzes the conversion of the substrate/ chromogen mixture to a colored product. After an incubation period, the reaction is stopped and stabilized by the addition of acid. Since the labeled carbendazim (conjugate) was in competition with the unlabeled carbendazim (sample) for the antibody sites, **the color developed is inversely proportional to the concentration of carbendazim in the sample.**

• Reagents

1. Carbendazim Antibody Coupled Paramagnetic Particles

The carbendazim antibody (rabbit anti-carbendazim) is covalently bound to paramagnetic particles, which are suspended in buffered saline containing preservative and stabilizers. A 65 mL volume is supplied in one vial.

2. Lyophilized Carbendazim Enzyme Conjugate

Concentrated horseradish peroxidase (HRP) labeled carbendazim analog is supplied as a lyophilized powder. One vial is provided.

3. Carbendazim Enzyme Conjugate Diluent

Buffered saline containing bovine serum albumin with preservative and stabilizers. A minimum of 35 mL is provided in one vial.

4. Carbendazim Standards

Three concentrations (0.25, 1.0, 5.0 ppb) of carbendazim standards in buffered saline with preservative and stabilizers are supplied. Each vial contains 2.0 mL.

5. Control

A concentration (approximately 2.5 ppb) of carbendazim in buffered saline with preservative and stabilizers. A 2.0 mL volume is supplied in one vial.

6. Diluent/Zero Standard

Buffered saline with preservative and stabilizers without any detectable carbendazim. A single vial containing 35 mL is provided.

7. Color Solution

A solution of hydrogen peroxide and 3,3',5,5'-tetramethylbenzidine in an organic base. A single vial containing 65 mL is provided.

8. Stopping Solution

A solution of sulfuric acid (0.5%). A single vial containing 60 mL is provided.

9. Washing Buffer

A 250 mL of buffered saline containing preservatives and stabilizers is provided.

10. Test Tubes

Polystyrene tubes (36) are packaged in a box.
100 test kit: three 36 tube boxes

• Reagent Storage and Stability

Store all reagents at 2-8°C. Do not freeze. Reagents may be used until the expiration date on the box. *The test tubes require no special storage condition and may be stored separately from the reagents to conserve refrigerator space.*

Consult state, local and federal regulations for proper disposal of all reagents.

• Materials Required but Not Provided

In addition to the reagents provided, the following items are essential for the performance of the test:

Pipets*	Precision pipets capable of delivering 200, 250 and 500 uL and a 1.0 mL repeating pipet.
Vortex Mixer*	Thermolyne Maxi Mix, Scientific Industries Vortex Genie, or equivalent

Magnetic Separation Rack*

RPA-I[™] RaPID Analyzer* or equivalent photometer capable of readings at 450 nm

* These items are available from Strategic Diagnostics Inc.

• Sample Information

This procedure is recommended for use with water samples. Other samples may require modifications to the procedure and should be thoroughly validated.

Samples containing gross particulate matter should be filtered (e.g. 0.2 um Anotop[™] 25 Plus, Whatman, Inc.) to remove particles.

Samples which have been preserved with monochloroacetic acid or other acids, should be neutralized with strong base e.g. 6N NaOH, prior to assay.

If the carbendazim concentration of a sample exceeds 5 ppb, the sample is subject to repeat testing using a diluted sample. A ten-fold or greater dilution of the sample is recommended with an appropriate amount of Diluent/Zero Standard or Sample Diluent. For example, in a separate test tube make a ten-fold dilution by adding 100 uL of the sample to 900 uL of Diluent/Zero Standard. Mix thoroughly before assaying. Perform the assay according to the Assay Procedure and obtain final results by multiplying the value obtain by the dilution factor e.g. 10.

The presence of the following substances up to 250 ppm were found to have no significant effect on Benomyl/Carbendazim RaPID Assay results: nitrate, sulfite, sulfate, copper, manganese, magnesium, mercury, calcium, thiosulfate and zinc. In addition, nickel and iron up to 100 ppm, silicates up to 500 ppm and sodium chloride concentrations up to 1 M showed no effect on results.

• Reagent Preparation

The Carbendazim Enzyme Conjugate is provided as a lyophilized preparation that must be reconstituted prior to use. To prepare the conjugate, add 3 mL of the conjugate diluent to the lyophilized conjugate vial. Swirl gently to dissolve the conjugate. Accurately transfer the vial contents to the diluent bottle. Repeat this procedure twice more with 3 mL aliquots of conjugate diluent. Invert diluent bottle several times to mix completely and let stand approximately 5 minutes before use. Enter the date of reconstitution on the side label of the Carbendazim Conjugate Diluent bottle. Also enter the expiration date of the Carbendazim Enzyme Conjugate solution which is **21 days** from the date of reconstitution. If the conjugate cannot be used within 21 days of reconstitution, aliquots should be prepared and frozen. *Frozen aliquots of reconstituted conjugate may be used until the expiration date found on the kit box label.*

All reagents must be allowed to come to room temperature and the antibody coupled paramagnetic particles should be mixed thoroughly before use.

• Procedural Notes and Precautions

As with all immunoassays, a consistent technique is the key to optimal performance. To obtain the greatest precision, be sure to treat each tube in an identical manner.

Add reagents directly to the bottom of the tube while **avoiding contact between the reagents and the pipet tip.** This will help assure consistent quantities of reagent in the test mixture.

Avoid cross-contaminations and carryover of reagents by using clean pipets for each sample addition and by avoiding contact between reagent droplets on the tubes and pipet tips.

Avoid foam formation during vortexing.

The magnetic separation rack consists of two parts: an upper rack which will securely hold the test tubes and a lower separator which contains the magnets used to attract the antibody coupled paramagnetic particles. During incubations the upper rack is removed from the lower separator so that the paramagnetic particles remain suspended during the incubation. **For separation steps, the rack and the separator are combined to pull the paramagnetic particles to the sides of the tubes.**

To obtain optimum assay precision, it is important to perform the separation steps carefully and consistently. Decant the rack by slowly inverting away from the operator using a smooth turning action so the liquid flows consistently along only one side of the test tube. While still inverted, place the rack on an absorbent pad and allow to drain. Lifting the rack and replacing gently onto the pad several times will ensure complete removal of the liquid from the rim of the tube (technique is demonstrated on training video, available from Strategic Diagnostics Inc.).

Mix the antibody coupled paramagnetic particles just prior to pipetting.

Do not use any reagents beyond their stated shelf life.

Avoid contact of Stopping Solution (sulfuric acid) with skin and mucous membranes. If this reagent comes in contact with skin, wash with water.

• Limitations

The Benomyl/Carbendazim RaPID Assay will detect benomyl, carbendazim and related compounds to different degrees. Refer to specificity table for data on several of the benzimidazole fungicides. The Benomyl/Carbendazim RaPID Assay kit provides screening results. As with any analytical technique (GC,

HPLC, etc...) positive results requiring some action should be confirmed by an alternative method.

The total time required for pipetting the magnetic particles should be kept to two (2) minutes or less, therefore the total number of tubes that can be assayed in a run should be adjusted accordingly.

• Quality Control

A control solution at approximately 2.5 ppb of carbendazim is provided with the Benomyl/Carbendazim RaPID Assay kit. It is recommended that it be included in every run and treated in the same manner as unknown samples. Acceptable limits should be established by each laboratory.

• Assay Procedure

Read Reagent Preparation, Procedural Notes and Precautions before proceeding.

1. Label test tubes for standards, control, and samples.

Tube Number	Contents of Tube
1,2	Diluent/Zero Standard, 0 ppb
3,4	Standard 1, 0.25 ppb
5,6	Standard 2, 1.0 ppb
7,8	Standard 3, 5.0 ppb
9	Control
10	Sample 1
11	Sample 2
12	Sample 3

2. Add 200 uL of the appropriate standard, control, or sample.
3. Add 250 uL of Carbendazim Enzyme Conjugate to each tube.
4. Mix the Carbendazim Antibody Coupled Paramagnetic Particles thoroughly and add 500 uL to each tube.
5. Vortex for 1 to 2 seconds minimizing foaming.
6. Incubate for 20 minutes at room temperature.
7. Separate in the Magnetic Separation Rack for **two (2) minutes**.
8. Decant and **gently** blot all tubes briefly in a consistent manner.
9. Add 1 mL of Washing Buffer to each tube and allow them to remain in the magnetic separation unit for **two (2) minutes**.
10. Decant and **gently** blot all tubes briefly in a consistent manner.
11. Repeat Steps 9 and 10 an additional time.
12. Remove the rack from the separator and add 500 uL of Color Solution to each tube.
13. Vortex for 1 to 2 seconds minimizing foaming.
14. Incubate for 20 minutes at room temperature.
15. Add 500 uL of Stopping Solution to each tube.
16. Add 1 mL Washing Buffer to a clean test tube. Use as blank in Step 17.
17. Read results at 450 nm within 15 minutes after adding the Stopping Solution.

• Results

Manual Calculations

1. Calculate the mean absorbance value for each of the standards.
2. Calculate the %B/Bo for each standard by dividing the mean absorbance value for the standard by the mean absorbance value for the Diluent/Zero Standard.
3. Construct a standard curve by plotting the %B/Bo for each standard on vertical logit (Y) axis versus the corresponding carbendazim concentration on horizontal logarithmic (X) axis on the graph paper provided.
4. %B/Bo for controls and samples will then yield levels in ppb of carbendazim by interpolation using the standard curve.

(Contact SDI for detailed application information on specific photometers.)

RPA-I RaPID Analyzer

Using the RPA-I RaPID Analyzer, calibration curves can be automatically calculated and stored. Refer to the RPA-I operating manual for detailed instructions. To obtain results from the Benomyl/Carbendazim RaPID Assay on the RPA-I the following parameter settings are recommended:

Data Reduct : Lin. Regression
Xformation : Ln/LogitB
Read Mode : Absorbance
Wavelength : 450 nm
Units : PPB
Rgt Blk : 0

Calibrators:
of Cals : 4
of Reps : 2

Concentrations:
#1: 0.00 PPB
#2: 0.25 PPB
#3: 1.00 PPB
#4: 5.00 PPB

Range : 0.10 - 5.00
Correlation : 0.990
Rep. %CV : 10%

• Expected Results

No interferences were observed in a study conducted on over 400 water samples from locations across the US using the Benomyl/Carbendazim RaPID Assay. When comparing the Benomyl/Carbendazim RaPID Assay to HPLC in a study of 15 samples, the methods were shown to correlate very well (r=0.991).

• Performance Data

Sensitivity

The Benomyl/Carbendazim RaPID Assay has an estimated minimum detectable concentration, based on a 90% B/Bo of 0.10 ppb.

Precision

The following results were obtained:

Control	1	2	3	4
Replicates	5	5	5	5
Days	5	5	5	5
n	25	25	25	25
Mean (ppb)	0.41	1.98	2.85	3.69
% CV (within assay)	13.5	7.2	5.7	6.4
% CV (between assay)	10.4	6.1	3.6	5.4

Recovery

Four (4) samples, including a municipal water source, drinking water from a local well, bay and creek water were spiked with various levels of carbendazim and then assayed using the Benomyl/Carbendazim RaPID Assay. The following results were obtained:

Amount of Carbendazim Added (ppb)	Mean (ppb)	S.D. (ppb)	Recovery %
0.5	0.55	0.15	110
1.0	1.05	0.11	105
3.0	2.95	0.18	98
4.5	4.22	0.35	94
Average			102

Specificity

The cross-reactivity of the Benomyl/Carbendazim RaPID Assay for various benzimidazole analogues can be expressed as the least detectable dose (LDD) which is estimated at 90% B/Bo, or as the dose required to displace 50% (50% B/Bo).

Compound	LDD (ppb)	50% B/Bo (ppb)
Carbendazim (MBC)	0.10	1.60
Benomyl	0.38	5.61
2-Benzimidazolylurea	0.62	9.22
Thiabendazole	6.30	91

Thiophanat-methyl	19.4	401
2-Aminobenzimidazole	120	2321
Benzimidazole	681	>10,000

The following compounds demonstrated no reactivity in the Benomyl/Carbendazim RaPID Assay at concentrations up to 10,000 ppb: alachlor, aldicarb, aldicarb sulfoxide, aldicarb sulfone, ametryn, atrazine, butylate, captofol, carbaryl, cyanazine, de-ethylatrazine, dicamba, 2,4-D, MCPA, mecoprop, metribuzin, pentachlorophenol, phosphamidon, picloram, procymidone, prometon, prometryn, propachlor, propazine, silvex, terbufos, terbutryn, terbutylazine and vinclozolin.

• Assistance

For ordering or technical assistance contact:

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Phone(800)544-8881
Fax(302)456-6782
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• Availability

Strategic Diagnostics Inc.
Benomyl/Carbendazim RaPID Assay
100 Test Kit
Benomyl/Carbendazim Sample Diluent

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